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Preservative effectiveness of essential oils in vapor phase combined with modified atmosphere packaging against spoilage bacteria on fresh cabbage

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A B S T R A C T

This study was conducted to investigate the antibacterial effects of various essential oils (EOs) against pathogens using the disc volatilization method. Also, combined effects of EOs in vapor phase and MAP were evaluated for reducing levels of total mesophilic microorganisms on fresh cabbage. The vapor phase activities of EOs (thyme-1, oregano-1, lemongrass-1, and lemongrass-2 oils) observed strong inhibitory effects. The MAP results showed that 100% CO_2 gas packaging reduced significantly levels of total mesophilic microorganisms on cabbage and radish sprouts, and their reduction level was 1.55 and 2.26 $\log_{10}$ CFU/g compared to control after 21 days of storage ($p \leq 0.05$). Based on previous results, combined effects of EOs in vapor phase and MAP (100% CO_2) showed that lemongrass-2 oil with 20 discs showed complete inactivation by <1.0 $\log_{10}$ CFU/g after 14 days of storage. These results could provide useful information for developing alternative preservation method to improve the freshness and shelf-life of fresh produce using natural antimicrobials.

1. Introduction

Consumers have an increased interest in fresh and health foods such as fruits and vegetables. However, fresh-cut fruits and vegetables deteriorate quickly and have limited shelf-life (Ghidelli, Mateos, Rojas-Argude, & Pérez-Gago, 2014). Major problems associated with fresh-cut fruits and vegetables during storage including tissue softening, discoloration, off-flavors, and off-odors are occurred because of microbial growth in the product (Gram et al., 2002; López-Gálvez, Peiser, Nie, & Cantwell, 1997). Also, foodborne outbreaks associated with consumption of raw vegetables have been increased (Santos et al., 2012). A number of studies have investigated foodborne pathogens isolated from raw vegetable (Beuchat, 1996; Nguyen-the & Carlin, 1994), including *Listeria monocytogenes* (Schlech III et al., 1983), *Salmonella* sp. (Doyle, 1990), and *Escherichia coli* (Nguyen-the & Carlin, 1994). Raw fresh produce could be contaminated with pathogens during harvesting through fecal material (manure, both of human and animal origin), human handling, washing procedure, processing equipment, transportation, and distribution (Beuchat, 1996; Johannessen, Loncarevic, & Kruse, 2002).

Many studies deal with washing chemical agents such as chlorine to inactivate pathogens on fruits and vegetables. Chemical agents are also used to extend shelf-life for minimally processed foods (Rodgers, Cash, Siddiq, & Ryser, 2004). Chlorine is widely used as a sanitizer for washing fruits, vegetables, and fresh-cut produce. It is commonly used at concentrations of 50–200 mg/L (Beuchat, 2008; Beuchat, Nail, Adler, & Clavero, 1998). However, it has limited effect in reduce level of microorganisms on fruits and vegetables (Beuchat, 1996). Moreover, chlorine may react with organic matter to form carcinogenic products in water (Parish et al., 2003). The inadequacy of chlorine as a sanitizer has stimulated interest in finding safer, more effective sanitizers (Ruiz-Cruz, Luo, Gonzalez, Tao, & González-Aguilar, 2006).

Recently, there has been an increasing consumer demands to replace chemically synthesized antimicrobial with natural antimicrobials for food preservation, such as essential oils (EOs) (Xu et al., 2007). EOs are considered generally regarded as safe (GRAS) and aromatic oily liquids extracted from various plant material as flowers, fruits, herbs, leaves, roots, seeds, and stem (Burt, 2004). EOs and some of their major components have shown antibacterial effects against pathogens and spoilage bacteria (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Burt, 2004; Holley & Patel, 2005). Several studies showed that EOs from spices and plants

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significantly decreased the levels of foodborne pathogens and food spoilage bacteria, especially *E. coli* O157:H7, *Shigella dysenteria*, *Bacillus cereus*, *Staphylococcus aureus*, *L. monocytogenes*, *Clostridium botulinum*, *Enterococcus faecalis*, *Staphylococcus* spp., *Bacillus* spp., enterobacteria, *Vibrio parahaemolyticus*, and *Pseudomonas fluorescens* (Burt, 2004; Ceylan & Fung, 2004; Pandit & Shelef, 1994; Ultee, Kets, & Smid, 1999). The antimicrobial activity of EOs in vapor phase has been demonstrated using direct contact methods (Delaquis, Ward, Holley, Cliff, & Mazza, 1999; Fisher & Phillips, 2006; Inouye, Takizawa, & Yamaguchi, 2001; López, Sánchez, Batlle, & Nerín, 2005; Suhr & Nielsen, 2003; Weissinger, McWatters, & Beuchat, 2001). However, the direct contact methods on food have been limited due to high hydrophobicity and volatility of the EOs (Nedorostova, Kloucek, Kokoska, Stolcova, & Pulkrabek, 2009).

Modified atmosphere packaging (MAP) is one of the most important preservation techniques used to maintain the quality and extend the shelf-life of foods (Jayas & Jeyamkondan, 2002; Martínez-Ferrer, Harper, Pérez-Muntoz, & Chaparro, 2002; Phillips, 1996). This technique replaces the air that surrounds the food in the package with a specific gas mixture, most commonly CO_2, O_2, and N_2 (Rao & Sachindra, 2002; Smigic et al., 2009). It can extend the shelf-life of perishable products such as meat, poultry, fish, fruits, and vegetables (Sandhya, 2010). The potential of MAP to prolong the shelf-life for many foods has been researched (Brecht, Chau, Fonseca, & Oliveira, 2001; Jacxsens, Devlieghere, & Debevere, 2001; Saltveit, 2003). Low levels of O_2 and high levels of CO_2 reduce the respiration rate, which in turn delays senescence, thus extending the storage time of the fresh produce (Saltveit, 1993).

However, there are very limited studies that investigated the antibacterial efficacy of EOs in fresh produce. The use of EOs as a food preservative may be limited, since the required high concentrations of EOs for food preservation cause changes in the organoleptic properties of food (Devlieghere, Vermeulen, & Debevere, 2004). Accordingly, low concentrations of EOs in vapor phase combined with physical or chemical treatments have been yet proposed. Developing an effective sanitization using natural antimicrobials against food spoilage microorganisms could result in an alternative preservation method to improve the freshness and shelf-life of fresh produce. Therefore, in this study we investigated the antibacterial effects of various EOs against pathogens and fresh produce spoilage bacteria using the disc volatilization method and MAP. We also combined effects of EO in vapor phase and MAP was evaluated for reducing the levels of total mesophilic microorganisms on fresh cabbage.

2. Materials and methods

2.1. Antibacterial effect of the EO treatments

2.1.1. Bacterial strains and growth conditions

Five strains of pathogens (*E. coli* O157:H7 ATCC 43895, *Salmonella* Typhimurium ATCC 19585, *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 4012, and *B. cereus* ATCC 10876) were obtained from the bacterial culture collection of Chung-Ang University (Anseong-si, Korea) and used in this study. All strains were maintained at −80 °C in 20% glycerol and were activated by cultivation in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI, USA, pH 7.3) for 24 h at 37 °C before use.

2.1.2. Preparation of EOs

EOs used in this study were purchased from Aromasavor (Seoul, Korea) and Whatsoap (Gwangju-si, Korea). In all cases, EOs were of the highest grade available (99–100% pure). The EOs was chosen on the basis of their potential in preservation of food produce against pathogens. The main components of the tested EOs are presented in Table 1.

2.1.3. Disc volatilization method

The disc volatilization method (López et al., 2005) was used to determine the diameter of the inhibition zone by various EOs against the 5 pathogens (*E. coli* O157:H7 ATCC 43895, *S.* Typhimurium ATCC 19585, *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 4012, and *B. cereus* ATCC 10876). Tryptic soy agar (TSA; Difco Laboratories, Detroit, MI, USA) was autoclaved at 121 °C for 15 min. Sterile medium (20 mL) was poured into a petri dish (90 mm diameter), and the plates were dried for 20 min in a safety cabinet. Bacterial cultures (0.1 mL) were inoculated onto the TSA and dried for 10 min in a safety cabinet. A sterile paper disc was laid on the inside surface of the upper lid, and 10 μl each of 100% EOs was pipetted onto the disc. The plates inoculated with pathogens were immediately inverted on top of the lid and sealed with parafilm to prevent leakage of EO vapor. The plates were incubated at 37 °C for 24 h. Antibacterial effect was evaluated by measuring diameters of the inhibition zones (mm) against tested pathogens.

2.2. Application of EOs on fresh produce

2.2.1. Modified atmosphere packaging (MAP)

Fresh cabbage and radish sprouts were purchased from a local supermarket (Anseong-si, Korea) and stored at 4 ± 2 °C before use. Fresh produce was washed with running tap water and dried for 20 min in a laminar flow biosafey hood. Samples (25 g) were placed in UV-sterilized plastic bags (NY bag, thickness 80 μm, polyethylene terephralate/aluminum/linear low-density polyethylene (PET/AL/L-LDPE), and O_2 permeability 23 cm^2/m^2 day atm at 23 °C; Gasung Pak Co., Gwangju-si, South Korea) and packed under 6 different atmosphere conditions: five different passive modified atmosphere (0% CO_2 and 100% N_2, 25% CO_2 and 75% N_2, 50% CO_2 and 50% N_2, 75% CO_2 and 25% N_2, 100% CO_2 and 0% N_2) and air packaging. Air packaging consisted of sealing without eliminating air in the bag with a vacuum packaging machine (AVS 200, CSE Any Vac, CSE Co.). The MAP gas mixture was prepared using a gas mixer (MAP Mix 9001 ME gas mixer, PBI Dansensor, Ringsted, Denmark). Bags were heat-sealed using a vacuum sealer (AZ-450E, Airzero, Ansansi, South Korea) connected to the gas mixer. Packed fresh produce were stored at 4 ± 2 °C for 0, 7, 14, and 21 days.

2.2.2. Disc volatilization method combined with MAP

From results of earlier part of this study, 4 EOs (thyme-1, oregano-1, lemongrass-1, and lemongrass-2) were chosen to combine with MAP on cabbage. Fresh cabbage was purchased from a local supermarket (Anseong-si, Korea) and stored at 4 ± 2 °C before use. Fresh cabbage was washed with running tap water and dried for 20 min in a laminar flow biosafey hood. Samples (25 g) were placed in UV-sterilized plastic bags (PET/AL/L-LDPE) and packed 100% CO_2 and 0% N_2 gas. And a ventilation-sterilized sachet with disc 1 piece, 10 pieces, and 20 pieces (10 μl/piece, 100% EOs) oil added into each packaging. For this, samples (25 g) were packaged in a sterilized stomacher bag with the combination of MAP and EOs, and then stored for 21 days at 4 ± 2 °C.

2.2.3. Gas composition measurement

The gas composition inside the packages was analyzed in each package before opening. The concentrations of CO_2 and O_2 in the